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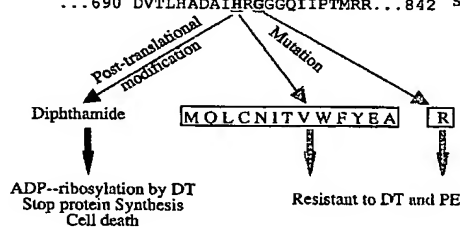
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(54) Title: METHODS FOR EXPRESSION AND PURIFICATION OF IMMUNOTOXINS

<i>H. sapiens</i> (66%)	...706	DVT	LHADAI	HRGGG	QIIPTARR...	858	SEQ ID NO:1
<i>M. musculus</i> (3'mRNA)	DVT	LHADAI	HRGGG	QIIPTARR...		SEQ ID NO:2
<i>R. norvegicus</i> (66%)	...706	DVT	LHADAI	HRGGG	QIIPTARR...	858	SEQ ID NO:3
<i>C. griseus</i> (CHO, 67%)	...706	DVT	LHADAI	HRGGG	QIIPTARR...	858	SEQ ID NO:4
<i>D. melanogaster</i> (67%)	...692	DVT	LHADAI	HRGGG	QIIPTARR...	848	SEQ ID NO:5
<i>C. elegans</i> (67%)	...700	DVT	LHADAI	HRGGG	QIIPTARR...	852	SEQ ID NO:6
<i>S. pombe</i> (79%)	...690	DVT	LHADAI	HRGGG	QIIPTARR...	842	SEQ ID NO:7
<i>P. pastoris</i> (88%)	...690	DVT	LHADAI	HRGGG	VIPTMKR...	842	SEQ ID NO:8
<i>S. cerevisiae</i>	...690	DVT	LHADAI	HRGGG	QIIPTMRR...	842	SEQ ID NO:9



(57) Abstract: In one aspect the present invention relates to a method of expressing an immunotoxin in *Pichia pastoris* strain mutated to toxin resistance comprising a) growing the *Pichia pastoris* in a growth medium comprising an enzymatic digest of protein and yeast extract and maintaining a dissolved oxygen concentration at 40% and above; and b) performing methanol induction with a limited methanol feed of 0.5-0.75 ml/min/ 10L of initial volume during induction along with a continuous infusion of yeast extract at a temperature below 17.5 °C., antifoaming agent supplied up to 0.07%, agitation reduced to 400 RPM, and the induction phase extended out to 163 h. In another aspect, the present invention relates to a method of purifying a nonglycosylated immunotoxin comprising a) loading a solution containing the nonglycosylated immunotoxin onto a hydrophobic interaction column; b) obtaining a first non-glycosylated immunotoxin containing eluant from the hydrophobic interaction column; c) loading the non-glycosylated immunotoxin containing eluant from step (b) onto an anion exchange column; d) obtaining a second

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CGG CGC GGA

GAT GTT ACC CTG CAC GCC GAT GCT ATC CAC AGA GGT GGA GGA CAA GTC ATT CCA ACC ATG AAG AGA

D V T L H A D A I H R G G G Q I I P T A R R

690 700 710

SEQ ID NO:10

non-glycosylated immunotoxin containing eluant from the anion exchange column by eluting the non-glycosylated immunotoxin with a sodium borate solution; e) diluting the concentration of sodium borate in the second non-glycosylated immunotoxin containing eluant from step (d) to about 50 mM or less; f) concentrating the diluted non-glycosylated immunotoxin containing eluant from step (e) over an anion exchange column; and g) obtaining a purified non-glycosylated immunotoxin from the anion exchange column.



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